Effect of Intermittent Oral 1,25(OH)₂D₃ Therapy on Bone Gla Protein in Dialysis Patients

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Abstract. Serum Bone Gla Protein (BGP) levels were measured by both immunoradiometric assay (IRMA) and radioimmunoassay (RIA) to investigate the effect of intermittent 1,25(OH)₂D₃ administration to dialysis patients who could not tolerate an increase in an active vitamin D₃ dose and/or calcium to control secondary hyperparathyroidism due to hypercalcemia. The administration of active vitamin D₃ gradually increased the serum BGP to more than 3 times the original level by the 8th week. At the 12th week after starting the active vitamin D₃ therapy, mean BGP was about twice the original level, which was about half the maximum level at the 8th week. The BGP (IRMA)/BGP (RIA) ratio was increased significantly at 4th and 8th weeks compared to the original level. During this period, serum calcium, phosphorous, or intact molecule PTH (I-PTH) levels showed insignificant changes, with a slight reduction in the mid molecule PTH (m-PTH) level, and a significant reduction in ALP. Serum BUN and creatinine levels were not changed significantly. These data suggest that BGP was increased through direct stimulation of osteoblasts by the active vitamin D₃, and the increase was not due to deterioration of secondary hyperparathyroidism. The reduction of the increase in the BGP level at the 12th week with insignificant biochemical changes suggests that activation of osteoblasts by vitamin D₃ may be transient. In conclusion, intermittent active vitamin D₃ increases serum BGP, without deterioration of major biochemical changes in patients with moderate to severe secondary hyperparathyroidism, although the increase may be transient. These facts suggest that the serum BGP of hemodialysis patients is controlled at least in part by active vitamin D₃. Whether the increase leads to histological amelioration of renal osteodystrophy or not remains to be determined.

Key words: Hemodialysis, BGP, Osteocalcin, Intermittent vitamin D₃.

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RENAL osteodystrophy is one of the major complications in patients under regular hemodialysis. The administration of active vitamin D₃, strict control of the serum phosphorous level, and the reduction of aluminum accumulation in the body are known to ameliorate renal osteodystrophy. Patients often have a higher serum calcium level after long-term hemodialysis, so that enough active vitamin D₃ and/or calcium cannot be administered to control the progression of secondary hyperparathyroidism. Active vitamin D₃ is known to reduce PTH synthesis [1], and its secretion [2]. Intermittent treatment with high dose active vitamin D₃ reduces the PTH level without causing severe hypercalcemia [3], and the treatment is widely accepted as “chemical parathyroidectomy”, although the treatment does not seem to be effective for patients with severe secondary hyperparathyroidism. Intermittent intravenous cal-
ciferol for 11.5 months histologically reduced bone metabolism in dialysis patients [3], but it is not stated how bone metabolism had been changed during the therapy. Frequent bone biopsy to investigate bone metabolism is clinically impractical, and other methods should be taken to investigate the changes in bone metabolism.

Among the parameters which reflect bone metabolism, serum bone Gla protein (BGP) is clinically acceptable. BGP, which is also called osteocalcin, is abundant noncollagenous protein and released into the blood stream from osteoblasts [4]. The serum level of BGP reflects bone formation even in dialysis patients [5]. As BGP synthesis depends on active vitamin D₃, and the administration of active vitamin D₃ stimulates BGP synthesis, an increase in serum BGP (RIA) following active vitamin D₃ was observed in dialysis patients [6]. But short-term effects of active vitamin D₃ on bone metabolism are not well known in moderate to severe secondary hyperparathyroidism.

Multiple immuno-reactive forms of BGP, 4 peaks in low turnover bone disease, and 6 peaks in high turnover bone disease including high molecular weight form, were identified in serum of patients with renal failure [7]. Therefore, falsely high BGP values may be obtained in patients under hemodialysis due to BGP fragments, if polyclonal antibody is utilized for the assay.

We investigated the effects of active vitamin D₃ on the BGP of dialysis patients, by both IRMA and RIA to measure serum BGP.

**Materials and Methods**

A dose of 4 µg/day of 1,25(OH)₂D₃ was administered twice/week to 10 patients under regular hemodialysis who could not tolerate any more vitamin D₃ and/or calcium supplement due to hypercalcemia. Five of them had not received vitamin D₃ administration, and the remaining 5 had been given a low dose of 1,25(OH)₂D₃ (0.25–0.5 µg/day) before this investigation. The mean age was 45.7±9.1 (Mean±SD) years old, and the mean dialysis period was 132±50 months. The observation period was 12 weeks, and blood samples were taken periodically during the investigation. The concentration of calcium in the dialysate was 3.0 mEq/l. The serum m-PTH level was measured with an HS-PTH assay kit provided by Yamasa Co., Ltd., (Chiba, Japan), and I-PTH was measured by the IRMA system of Allegro, (Nichols Institute, CA, USA).

The serum BGP level was measured with an RIA kit provided by Yamasa Co., Ltd., (Chiba, Japan) with a normal value of 7.4±2.2 ng/ml, and by an IRMA system [8] using a monoclonal antibody to both human BGP (12–33) and BGP (34–49), (Mitsubishi Petrochemical Co., Ltd., Ibaraki, Japan) with a normal value of 7.8±3.6 ng/ml ranging from 3.9 ng/ml to 12.1 ng/ml. Samples with extremely high value were diluted with zero standard serum. The coefficient of variation of intra-assay was 2.2–2.4%, and that of inter-assay was 2.2–5.2% with 104–116% recovery including a sample with a high concentration at 103.3 ng/ml. A sample obtained from a dialysis patient was fractionated according to the procedure described by Gundberg [7], and two peaks were found. The value at the intact BGP molecule position showed 78.3% of total BGP (IRMA). Statistical significance was determined by ANOVA, and paired t-test. The institutional review board approved the experiment, and the experimental design was explained to all the patients.

**Results**

Four patients showed transient hypercalcemia, and the dose of vitamin D₃ was temporarily reduced, although most of them could tolerate 4 µg of 1,25(OH)₂D₃ twice a week. One patient was omitted after the 4th week due to gastrointestinal bleeding, and the other declined to take vitamin D₃ due to itching after the 8th week. The frequency of hypercalcemia (serum calcium > 5.4 mEq/l) versus the number of measurements in these patients was 6.9–10.8%. Serum calcium was insignificantly increased from 4.72±0.24 mEq/l at the beginning of the investigation to 4.80±0.29 mEq/l at the 12th week. The frequency of hypercalcemia (serum calcium > 5.4 mEq/l) versus the number of measurements in these patients was 6.9–10.8%. Serum calcium was insignificantly increased from 4.72±0.24 mEq/l at the beginning of the investigation to 4.80±0.29 mEq/l at the 12th week. Serum phosphorous was slightly increased during period of the experiment from 4.63±0.18 mg/dl to 5.38±1.15 mg/dl, but the difference was not significant (Fig. 1). ALP was gradually decreased from 951.7±407.0 IU to 534.1±401.8 IU at the 12th week (Table 1). The change was statistically significant (p<0.01 by ANOVA). The mean m-PTH level was 118.1±54.3 ng/ml before the therapy and was insignificantly reduced to 85.5±25.6 ng/ml at the
Fig. 1. Serum calcium and phosphorous were measured every 2 weeks during the investigation. These values at the 12th week were not significantly different from the initial values in a paired t-test. Open circles show serum calcium levels, and open triangles show serum phosphorous levels.

Table 1. Changes of ALP and PTH

<table>
<thead>
<tr>
<th></th>
<th>ALP (IU)</th>
<th>m-PTH (ng/ml)</th>
<th>I-PTH (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 Week</td>
<td>951.7±407.0</td>
<td>118.1±54.3</td>
<td>755.5±232.7</td>
</tr>
<tr>
<td>2 Week</td>
<td>986.7±423.2</td>
<td>103.9±40.0</td>
<td>871.1±264.8</td>
</tr>
<tr>
<td>4 Week</td>
<td>883.9±417.6</td>
<td>105.2±39.4</td>
<td>949.7±207.1</td>
</tr>
<tr>
<td>8 Week</td>
<td>750.0±457.8</td>
<td>92.8±47.9</td>
<td>750.1±260.4</td>
</tr>
<tr>
<td>12 Week</td>
<td>534.1±401.8*</td>
<td>85.5±25.6</td>
<td>618.7±297.3</td>
</tr>
</tbody>
</table>

ANOVA  p<0.01  0.05<p<0.1  p>0.5

ALP was significantly reduced (p<0.01) and m-PTH showed a tendency to decrease (0.05<p<0.1), but I-PTH was not significantly changed (p>0.1) by the ANOVA method. * shows p<0.05 vs. 0 week.

12th week. The serum I-PTH level was also insignificantly changed from 755.5±232.7 pg/ml to 618.7±297.5 pg/ml at the 12th week (Table 1).

Serum BGP measured by RIA was 73.3±31.6 ng/ml before the treatment, 108.3±54.7 ng/ml at the 2nd week, 149.3±53.0 ng/ml at the 4th week, 214.2±63.4 ng/ml at the 8th week and 122.6±41.6 ng/ml at the 12th week (Fig. 2). The BGP level measured by IRMA was significantly increased from 49.6±23.9 ng/ml to 83.2±34.3 ng/ml at the 2nd week, 148.0±53.0 ng/ml at the 4th week, 185.4±75.7 ng/ml at the 8th week and 116.9±62.5 ng/ml at the 12th week, respectively (Fig. 2). The individual time course for BGP (IRMA) is shown in Fig. 3. The increases in BGP detected by RIA or IRMA were statistically significant (p<0.01 by ANOVA). The degree of the increase was not affected by the low dose of active vitamin D3 prior to this investigation. The BGP (IRMA)/BGP (RIA) ratio was increased from 66.9±9.6% before this study, to 82.4±26.2% at the 2nd week, to 91.2±21.5% at the 4th week, to 86.0±18.9% at the 8th week, and to 95.4±39.8% at the 12th week (Fig. 4). The increases at both the 4th week and 8th week were statistically significant (p<0.05, by paired t-test). In contrast, neither serum BUN nor creatinine was changed significantly during the experimental period.
Discussion

BGP is synthesized in osteoblasts, released into the blood stream, and metabolized by the kidneys [9]. The serum BGP level is one of the markers of osteoblastic function, and correlates with bone turnover [5]. The level is reported to be increased in patients with hyperparathyroidism or renal failure. Serum BGP increases with age, probably due to the increased frequency of fracture by osteoporosis or the increase in bone turnover after menopause [10], but other groups reported that the level decreased with age in both sexes [11]. Judging from the high BGP levels in dialysis patients, the sex or age differences would be so small that they can be ignored in the evaluation of bone metabolism in adult dialysis patients.

The intravenous administration of calcitriol to patients with refractory ostitis fibrosa secondary to chronic renal failure is effective in reducing degree of secondary hyperparathyroidism and in ameliorating bone changes [3]. In the previous report, the serum BGP level was increased after long-term 1-alpha(OH)D3 therapy [7], but short-term change in BGP was not reported in that paper.

To evaluate bone metabolism sequentially, the measurement of the serum BGP is clinically useful. BGP can be measured easily by RIA. As the serum BGP of dialysis patients measured by RIA consists of at least 4 to 6 different molecules including fragments [7], the serum BGP level of the intact molecule form may reflect the real bone metabolism in dialysis patients. As commercially available RIAs usually utilize the cross reaction with antibody to cow BGP, the serum level may be inappropriately high, and mislead in the estimation of bone metabolism in dialysis patients, because of a cross reaction with BGP fragments. The monoclonal antibodies utilized for IRMA in the present study were reactive with BGP (34–49) and BGP (12–33), and the BGP (IRMA) levels would more precisely show the bone metabolism of these patients. The significant increase in serum BGP due to the treatment proved that osteoblasts of dialysis patients can be stimulated for 8 weeks, followed by a reduction in the stimulation. The increase in BGP with insignificant changes in the PTH level and/or renal function suggests that the increase in bone metabolism was not caused by the deterioration of secondary hyperparathyroidism or renal function. The significant increase in the ratio of BGP (IRMA) to BGP (RIA) suggests that the increase in the BGP level was due to increased formation of intact BGP by osteoblasts, although a reduction in BGP metabolism cannot be ruled out completely.

BGP is a vitamin D dependent protein and active vitamin D3 increased BGP production in an in vitro study [12]. Although the serum BGP level correlates with the serum PTH level, evidence that PTH directly stimulates BGP production in vivo is lacking. Firstly, PTH suppresses BGP production in vitro [12]. Secondly, active vitamin D3 increases BGP independently of the PTH level in both iideopathic hypoparathyroidism and pseudohypo-parathyroidism [13]. Lastly, the high BGP level in primary hyperparathyroidism may be due to the increase in 1,25(OH)2D3 rather than the high PTH level. These facts suggest that the serum BGP level is controlled at least in part by active vitamin D3 in patients with normal renal function. But the regulation of serum BGP in dialysis patients is not well understood, although the increase in bone formation and/or reduction in BGP metabolism by the kidney are suggested as causes of the high BGP level. Intermittent 1,25(OH)2D3 therapy increased serum BGP with insignificant biochemical changes, including the PTH level in the present study, suggesting that the serum BGP in dialysis patients is also controlled by 1,25(OH)2D3. The difference between changes in BGP and ALP may be explained in part by increased BGP production in osteoblasts due to active vitamin D3 treatment and/or a reduction in the ALP increase due to amelioration of secondary hyperparathyroidism, although the reason for the
difference could not be determined in this investigation.

The reason for the reduction in the BGP increase at the 12th week was not clear. The reduction of the vitamin D3 dose, followed by a decrease in BGP production, was not the main reason, because the mean vitamin D3 dose was not changed during the investigation. Down regulation of the vitamin D3 receptor of osteoblasts is not a reasonable explanation, because vitamin D receptors are usually up-regulated both in vitro [14, 15] and in vivo [16]. Amelioration of renal osteodystrophy may be one explanation.

During the investigation, mild hypercalcemia occurred. Although hypercalcemia could be controlled easily by temporary reduction of the vitamin D3 dose, the incidence or degree of ectopic calcification might be increased during long term treatment. Therefore, the clinical value of intermittent treatment of dialysis patients with active vitamin D3, especially long-term treatment, remained to be determined.

References